



Europäisches Patentamt
European Patent Office
Office européen des brevets

(11) Publication number:

0 385 539
A2

(12)

EUROPEAN PATENT APPLICATION

(21) Application number: 90200422.5

(51) Int. Cl. 5: A01J 7/00, A01J 5/04,
A01J 5/08

(22) Date of filing: 23.02.90

(30) Priority: 27.02.89 NL 8900479

(43) Date of publication of application:
05.09.90 Bulletin 90/36

(84) Designated Contracting States:
AT BE CH DE DK FR GB IT LI SE

(71) Applicant: C. van der Lely N.V.
Weverskade 10 P.O. Box 26
NL-3155 ZG Maasland(NL)

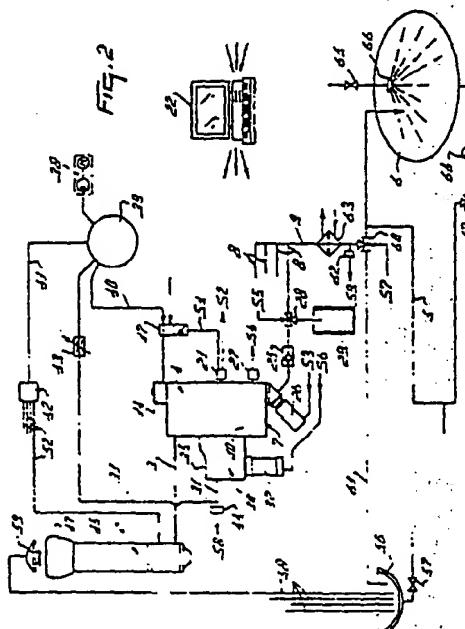
(72) Inventor: van der Lely, Edwin
10A Weverskade
NL-3155 PD Maasland(NL)
Inventor: van den Berg, Karel
5 Boterbloemstraat
NL-2971 BR Bleskensgraaf(NL)
Inventor: Fransen, René
6 Prins Hendriklaan
NL-3135 ZD Vlaardingen(NL)

(74) Representative: Mulder, Herman et al
Octrooibureau Van der Lely N.V. Weverskade
10 P.O. Box 26
NL-3155 ZG Maasland(NL)

(54) A milking plant

(57) A milking plant for cows is provided with a milking machine comprising teat cups (2), which can be connected separately to the teats of an animal. The milk obtained from each udder quarter is conveyed through a separate line (3) to a milk measuring device (4). The milk measuring device (4) includes four milk meters (7), whose separate discharge lines (8) are coupled to a common discharge line (9) terminating in a milk cooling tank (6). The milking plant further comprises a rinse line system (56, 57, 58, 59, 2, 3, 7, 8, 9, 60, 61) and a circular line (5), to which the discharge lines (9) of the milk measuring devices (4) are connected and which circular line (5) comprises the cooling tank (6).

EP 0 385 539 A2



A MILKING PLANT

The present invention relates to a milking plant for milking cows, which plant comprises teat cups that are connectable to the respective teats of a cow's udder, while the milk obtained from each udder quarter with the aid of the said teat cups can be conveyed through a separate line to a milk measuring device.

In prior art milking plants of the above-defined type, the teat cups are combined in one single milking claw, while the buffer room in the milking claw, to which room the teat cups are connected directly, comprises four separate chambers. Via four separate milk hoses the milking claw is connected to a milk meter, from which the milk can be separated or be supplied to a milk cooling tank. The four-quarter milking claw has for its object to provide a better means to establish the health of the udder; the milk originating from an inflamed quarter has a higher conductivity than that from a healthy one. The electric conductivity of the milk can be measured for each quarter, whereafter it can be determined whether the milk must be separated or not.

Such a structure of the milking plant is not suitable when no use is made of one single milking claw, but of a milking system wherein the teat cups are connected individually and independently of each other, e.g. by a milking robot, to the teats of the udder..

Therefore, the invention has for its object to provide a milking plant which is not only suitable for use when one single milking claw is used, but also in those cases wherein the teat cups are connected individually and independently of each other.

According to the invention, the milking plant of the above-defined type is characterized in that the milk measuring device includes four milk meters, whose separate discharge lines are coupled to a common discharge line terminating in a milk cooling tank. Due to the largely parallel structure of the milking system, there is effected an improved check on the milk production. The milk production can be effected from each udder quarter individually, while the teat cups can be removed independently of each other; consequently, the teats will not be burdened unnecessarily.

In addition, according to the invention, a milk meter includes a milk receptacle and a measuring chamber, in which connection the milk flows under a vacuum from the milk receptacle into the measuring chamber and is pumped in defined quantities by means of compressed air from the measuring chamber into the relevant, separate discharge line. In particular, according to the inven-

tion, the air sucked along by the vacuum together with the milk from the relevant teat cup is separated therefrom in the milk meter. Consequently, milk containing air is passed from the teat cup into the milk meter, whereas it is only the milk that is discharged therefrom to the milk cooling tank; in this respect it should be noted that so far it usually has been the custom to effect the milk-air separation not earlier than in the milk cooling tank. In a specific embodiment in accordance with the invention, the milk meter is provided with a valve rod which, in a first position under a vacuum, leaves the aperture between the milk receptacle and the measuring chamber open, and, in a second position by means of compressed air, closes same. More specifically, in accordance with the invention, there is provided a switching element, with the aid of which, in a first switching position thereof, the vacuum is applied to cause the valve rod to be brought to its first position, and, in a second switching position thereof, compressed air is admitted to cause the valve rod to be brought to its second position. Furthermore, according to the invention, the measuring chamber is provided with a milk level sensor, by means of which it is established when a defined quantity of milk is contained therein, after which it produces a control signal to cause the switching element to be adjusted from its first position to its second position. Also, in accordance with the invention, the switching element may be adjusted from its second position to its first position after a fixed period of time. However, this might also be effected by means of a sensor which senses that all the milk has been pumped from the measuring chamber, or by means of a flow sensor provided in the line beyond a teat cup. Furthermore, according to the invention, each time when a defined quantity of milk has been established in the measuring chamber, the milk level sensor may produce a control signal intended for a computer, in which the quantity of milk obtained from each udder quarter is recorded for each individual cow. In particular, the recording of this quantity and hence also that of the total quantity of milk obtained from each cow per unit of time, e.g. per-day, may be kept on the basis of a progressive average calculated over a defined number of days.

According to the invention, the measuring chamber, when empty, is closed by a spherical body. Preferably, this spherical body is of such a design that, when the milk flows from the milk receptacle into the measuring chamber, it floats on the milk contained therein. Hereby it is achieved that each time a predetermined quantity of milk contained in the measuring chamber is pumped off

by means of the compressed air admitted thereto for the purpose. Furthermore, according to the invention, a non-return valve is included in the separate discharge pipe beyond the aperture in the measuring chamber, which aperture can be closed by the spherical body. Via this non-return valve the milk is passed from the measuring chamber, but only under the influence of compressed air. In the opposite direction, the non-return valve blocks any milk flow, which might be possible in case the pressure in the relevant discharge pipes would exceed that prevailing in the measuring chamber in front of the non-return valve. This situation might be conceivable, since the milk is discharged from four milk meters to the milk cooling tank through the same common discharge line. The presence of the non-return valve beyond the measuring chamber aperture that is closable by the spherical body implies that, during the milking operation, the first obtained milk fills the separate discharge pipe between the said aperture and the non-return valve, as a result of which, according to the invention, each time a cow is milked, the quantity of milk recorded in a computer is increased only once by the quantity of milk corresponding to the volume of the separate discharge pipe between the measuring chamber aperture that is closable by the spherical body and the non-return valve.

In order to ensure the admission of compressed air via the measuring chamber into the discharge lines, in particular when these lines must be cleared of milk thereby e.g. in connection with a subsequent rinsing procedure, in accordance with the invention, the milk meter is provided with a lifting magnet which, by moving the spherical body, has for its object to establish a connection between the closed measuring chamber and the discharge lines.

According to the invention, each milk meter of the milk measuring device is provided with a milk conductivity sensor. More in particular, the milk conductivity sensor is arranged in the measuring chamber of a milk meter. The conductivity of the milk is a measure of the health of the udder. The milk originating from an inflamed quarter has a higher conductivity than that from a healthy one. The milk originating from an inflamed quarter must be separated from the rest of the milk. According to the invention, a separate discharge line is provided with a three-way valve, through which the milk pumped from a relevant milk meter is either discharged into the common discharge line or, after it has been found that the milk originates from an inflamed quarter, into a receptacle specially provided for the purpose. This three-way valve may be operated automatically as soon as a relevant milk conductivity sensor has measured a conductivity which exceeds a preset fixed value. In a preferred

embodiment in accordance with the invention, however, the milk conductivity sensor applies a control signal to a computer, in which it is determined whether or not the relevant milk originates from an inflamed quarter, after which, when the former appears to be the case, the computer supplies a control signal to the three-way valve in the relevant separate discharge line in order that the milk in question is discharged to the receptacle. The conductivity of the milk from the individual quarters can be compared with each other in the computer, whereby it is possible to establish from the spread in the conductivity values a potential inflammation of one of the quarters. Since older cows and cows in late lactation inherently have a higher milk conductivity than younger and freshly calved ones, it will be better not to compare deviations in conductivity to a preset fixed value, but to compare the deviations to the cow's own standard, i.e. to compare the conductivity in the computer with a progressive average determined over a number of days for the relevant cow. The calculation of the progressive average may, of course, be combined with the afore-mentioned calculation of the spread.

According to the invention, a milk meter is provided with a tube stop valve, with the aid of which the vacuum connection of the milk meter can be closed after milking. The end of the milking procedure can be detected by means of sensors, e.g. with the aid of a milk flow sensor which may be incorporated in the line between the teat cup and the milk meter or in the milk meter itself, or with the aid of the afore-mentioned milk level sensor. In accordance with the invention, it is likewise possible to activate the tube stop valve after a milking period of a duration laid down in a computer, e.g. on the basis of the number of times the measuring chamber has been emptied, has ended, i.e. by applying thereto a control signal supplied by the computer. In a specific embodiment, the tube stop valve includes a lifting mechanism to allow air to flow into the milk meter simultaneously with or immediately after closure of the vacuum connection of the measuring chamber. This can be effected with a certain amount of overpressure; a simpler method is to have the lifting mechanism be operated, in accordance with the invention, by a valve in the outer wall of the milk meter. The tube stop valve in accordance with the invention renders it possible, in a simple manner, to remove the vacuum in a teat cup before the teat cup is removed from the teat or drops down therefrom.

The milking plant is provided in customary manner with a vacuum pump having a vacuum balance tank to increase the vacuum stability. For larger plants in particular, preferably, the pump and the balance tank are arranged remote from each other and the balance tank is positioned close to

the milking parlour. The balance tank has a plurality of vacuum connections: for each milk measuring device four connections for the vacuum connection of the individual milk meters, four connections for the valve rod control in each milk meter and a connection for an electronic pulsator system for the four teat cups. A separate pulsator may be provided for each teat cup. The suction-rest ratio in the pulsator system may be adjustable, i.e. that for the rear quarters may be adjusted independently of that for the front quarters. According to the invention, the suction-rest ratio can be set by a computer in dependence on the milk flow. When the milk flow decreases, it will be possible to adjust a comparatively longer rest. This contributes to a reduction in the burdening of the teats, which is of the utmost importance when the animal is milked several times a day. According to the invention, a throttle ring is included in the vacuum connection from the balance tank to a milk meter. This ring prevents, should one of the teat cups fall from a teat, fluctuations from occurring in the prevailing vacuum and more particularly in the vacuum in the other teat cups. So as to ascertain whether a vacuum is present in a milk meter, in accordance with the invention, there is provided an air flow sensor in the vacuum connection between the throttle ring and the milk meter.

In the situation wherein the four teat cups are incorporated in one single milking claw, the claw housing, whether or not divided into four compartments, constitutes a buffer room for the milk transport and for counteracting fluctuations in the vacuum under the teats to which the teat cups are connected. In order to obtain the same result in the situation wherein the teat cups are not incorporated in one single milking claw, but are connected to an associated milk meter which is in a relatively more remote position, in accordance with the invention, a teat cup is provided with such a buffer room at its lower end. In particular, this buffer room for the milk transport is in connection with the outer air via an air suction aperture. In connecting the teat cups, it is optionally possible, in order to facilitate this connection, to employ an increased vacuum, although in that case more air is sucked in.

It must be possible for the milk line system to be rinsed after milking. In accordance with the invention, for this purpose there is included in the common discharge line a three-way valve, through which in a first position thereof the milk is passed via the common discharge line to the milk cooling tank, whilst in its second position the three-way valve establishes a rinse line system. In particular, the rinse line system includes a rinse jetter which, with the object of flushing the lines, is connected to a teat cup, while a rinsing fluid is passed by the vacuum in a milk meter from a rinsing fluid con-

tainer through a first rinsing line to the rinse jetter and from there, via a teat cup, into the relevant milk meter, whereafter the rinsing fluid is returned through the relevant separate discharge line, the common discharge line, the three-way valve incorporated therein, and a second rinsing line to the rinsing fluid container. Prior to rinsing, all the milk must first have been forced from the lines to be rinsed, in particular all the milk present in the common discharge line must have been forced through the three-way valve incorporated therein. In order to be able to establish that this has been done indeed, according to the invention, an air-milk sensor is provided in the common discharge line in front of the three-way valve incorporated therein.

Furthermore, in accordance with the invention, a heat exchanger is provided in the common discharge line in front of the three-way valve incorporated therein as a pre-cooling device for the milk cooling tank.

In accordance with the invention, the milk cooling tank may be included in a circular line, to which the common discharge lines of the individual milk meters are connected. More generally, therefore, the invention relates to a milk measuring system for milking cows, which system comprises a milk cooling tank, characterized in that this milk cooling tank is included in a circular line, the discharge lines of the individual milk meters being connected to the said circular line. In order to prevent that, after milking, residual milk remains in the circular line, in accordance with the invention, the circular line is provided with a pump to pump the milk from the milk cooling tank therethrough. Preferably, the circular line should be thermally insulated. The milk being circulated from the milk cooling tank, the means for keeping same in motion thereinside have become superfluous. In a preferred embodiment in accordance with the invention, the milk cooling tank is provided with a rinsing fluid supply line, through which, after all the milk has been removed from the milk cooling tank, a rinsing fluid can be introduced into the milk cooling tank, which rinsing fluid is circulated through the circular line by means of the pump. In particular, the pump can operate at two speeds, i.e. the milk is circulated at a relatively low speed and the rinsing fluid at a relatively high speed. Furthermore, in accordance with the invention, a three-way valve is incorporated in the circular line, by means of which the circulating rinsing fluid can be discharged. In order to perform the rinsing of the milk cooling tank in a most advantageous manner, in accordance with the invention, the milk cooling tank includes a sprayer connected to the rinsing fluid supply line; by mounting this sprayer in such a way that it is movable, so that the entire inner surface of the milk cooling tank can be covered thereby, an extremely

efficient rinsing system is obtained.

For a better understanding of the invention and to show how the same may be carried into effect, reference will now be made, by way of example, to the accompanying drawings, in which:

Figure 1 shows schematically an arrangement of a milking plant for milking cows;

Figure 2 shows a basic arrangement of the milking plant in accordance with the invention;

Figure 3 is a more detailed representation of the basic structure of a milk meter in the arrangement shown in Figure 2, and

Figure 4 is a more detailed representation of the basic structure of a teat cup in the arrangement shown in Figure 2.

Corresponding components in the drawings have been denoted by the same reference numerals.

Figure 1 shows a schematic arrangement of a milking plant for milking cows. Only two milking parlours are shown by way of illustration, in each of which a cow is present. For each milking parlour there is provided a milking robot 1 having four teat cups 2 at its end. For the matter of that, the invention is completely independent of the manner in which the teat cups are connected; the teat cups may, combined in one single milking claw, be connected together as well as individually and independently of each other to respective teats of a cow's udder. The milk obtained from each udder quarter with the aid of the teat cups 2 can be conveyed through a separate line 3 (see Figure 2) to a milk measuring device 4. From the milk measuring device 4 the milk is conveyed via a circular line 5, to which the discharge lines of the various milk measuring devices in the various milking parlours are connected, to a milk cooling tank 6.

The milk measuring device 4 comprises four milk meters 7, only one of which is shown in Figure 2. Figure 2 furthermore illustrates the basic arrangement of the milking plant, only one teat cup 2 and only one milk meter 7 having been shown in this arrangement for the sake of simplicity. The individual discharge lines 8 of the milk meters 7 are coupled to a common discharge line 9, which terminates in the milk cooling tank 6. Figure 3 is a more detailed representation of the basic structure of a milk meter as is incorporated in the milking plant shown in Figure 2. The milk meter 7 includes a milk receptacle 10 and a measuring chamber 11, in which connection the milk flows under a vacuum via the separate line 3 connected to the teat cup 2 from the milk receptacle 10 into the measuring chamber 11 and is pumped in defined quantities by means of compressed air from the measuring chamber 11 into the separate discharge line 8. The milk meter 7 includes a valve rod 12 which, in a first (shown) position under a vacuum, leaves the

aperture 13 between the milk receptacle 10 and the measuring chamber 11 open, and, in a second (non-shown) position by means of compressed air, closes same. At its upper side, the milk meter includes a chamber 14, in which chamber the valve rod 12 has a piston 15. The valve rod 12 is capable of upward and downward movement in and through this chamber 14. In the space below the piston 15 there is provided in the wall of the chamber 14 an aperture 16. Via this aperture 16, the milk meter 7 is connected to a switching element 17 (see Figure 2). In a first position of this switching element 17, a vacuum is applied to cause the valve rod 12 and the piston 15 to be moved downwardly to its first position, thereby leaving the aperture 13 open. In a second position of this switching element 17, compressed air is admitted into the space below the piston 15 to cause the valve rod 12 and the piston 15 to be moved upwardly to its second position, thereby closing the aperture 13. The milk meter 7 furthermore includes a tube 18 which, via relatively narrow apertures 19 and 20, is connected to the space in the chamber 14 below the piston and to the measuring chamber 11, respectively. When compressed air is admitted into the space below the piston 15, the aperture 13 is closed immediately and the air is forced into the measuring chamber 11 via the aperture 19, the tube 18 and the aperture 20, as a result of which the milk present in the measuring chamber 11 is passed into the separate discharge line 8.

The measuring chamber 11 is provided with a milk level sensor 21, by means of which it is established when a defined quantity of milk is contained therein. When the milk level in the measuring chamber 11 has reached that of the sensor 21, this sensor 21 supplies a control signal S1 (see Figure 2) to cause the switching element 17 to be adjusted from its first position to its second position, so that the measuring chamber 11 can be emptied. After a fixed period of time, the switching element 17 is adjusted from its second position to its first position; this period of time is of such a duration that there is sufficient time for the measuring chamber 11 to be emptied. When the switching element 17 has returned to its first position, the measuring chamber 11 can be filled again. The milk level sensor 21 can also apply a control signal S2 to a computer 22, in which the quantity of milk obtained from each udder quarter is recorded for each individual cow.

In the lower part of the measuring chamber 11 there is provided a spherical body 23. When the measuring chamber 11 is empty, this spherical body 23 closes the aperture 24 between the measuring chamber 11 and the separate discharge line 8. The spherical body 23 is made of such a material that, when the milk flows from the milk recep-

tacle 10 into the measuring chamber 11, it floats on the milk contained therein. When the milk is forced from the measuring chamber 11 into the separate discharge line 8, the aperture 24 is closed automatically by the spherical body 23 once the measuring chamber 11 is empty. A non-return valve 25 is arranged in the separate discharge line 8 beyond the aperture 24 in the measuring chamber, preferably as closely as possible therebeyond. Via this non-return valve 25 the milk is allowed to pass from the measuring chamber 11 but only under the influence of compressed air. In the opposite direction, the non-return valve 25 blocks any milk flow which might be possible in case, for whatever reason, the pressure in the relevant discharge lines would exceed that prevailing in the measuring chamber 11 in front of the non-return valve 25. When, during the milking operation, the first obtained milk flows into the measuring chamber 11, then not only the measuring chamber 11 itself will be filled, but also the space in the separate discharge line 8 between the non-return valve 25 and the said aperture 24. By each subsequent pump stroke it is only the milk contained in the measuring chamber that is pumped off, so that the total quantity of milk as determined by the computer during milking for each udder quarter must be increased only once by the quantity of milk corresponding to the volume of the separate discharge line 8 between the non-return valve 25 and the said aperture 24. This is, however, a constant correction to be entered into the computer 22 on recording of the quantity of milk obtained. After milking, the milk must also be forced from the separate discharge lines 8 and the common discharge line 9, i.e. into the circular line 5 to the milk cooling tank 6. For that purpose, the aperture 23 in the measuring chamber 11 must be free so as to allow compressed air to pass. To that end, the milk meter 7 is fitted with a lifting magnet 26 which moves the spherical body 23 upwardly in response to a control signal S3 supplied by the computer 22.

The milk meter 7 includes a milk conductivity sensor 27, which sensor, preferably, is arranged in the measuring chamber 11. The control signal S4 supplied by the milk conductivity sensor 27 is a measure of the health of the udder; in the case of mastitis, the conductivity of the milk is higher than that of the milk obtained from a healthy udder or from a healthy udder quarter. The milk originating from an inflamed udder quarter must be separated off. For this purpose, the separate discharge line 8 is provided with a three-way valve 28, through which the milk pumped from the milk meter 7 is either passed into the common discharge line 9 or, after it has been found that the milk originates from an inflamed quarter, into a receptacle 29 specially provided for the purpose. The three-way valve 28

might be operated automatically as soon as the signal S4 produced by the milk conductivity sensor 27 indicates a value which exceeds a preset value. It is, however, more advantageous to apply the control signal S4 to the computer 22 which, taking account of the further conditions of the specific cow, supplies a control signal S5, by means of which the three-way valve 28 can be operated.

In addition, the milk meter 7 is provided with a tube stop valve 30, with the aid of which the vacuum connection 31 can be disconnected from the milk meter after milking. During milking, a vacuum prevails in the milk receptacle 10. After the milking procedure has ended, the vacuum must be removed, and it is not until then that the teat cups are removed from the teats. Prior to removing the vacuum from the milk receptacle 10, the vacuum line 31 must be closed first. For that purpose, after a milking period preset in the computer 22 has elapsed, a control signal S6 is applied to the electromagnet 32 of the tube stop valve 30. By means of the then energized electromagnet 32, a rod 33 having a spherical end 34 is moved upwardly to seal the vacuum connection 31 against the fixed stop 35. With the aid of a lifting mechanism 36 which is connected pivotably to the rod 33, a valve 37 in the wall of the milk receptacle 10 is drawn upwards simultaneously, as a result of which air can flow freely thereto.

The milking plant as shown schematically in Figure 2 includes in a customary manner a vacuum pump 38 having a vacuum balance tank 39 to increase the vacuum stability. The balance tank 39 has a plurality of vacuum connections. A vacuum connection 31 is provided for the milk receptacle 10 of each milk meter 7. A vacuum connection 40 is present for each switching element 17. In addition, a vacuum connection 41 is provided for an electronic pulsator system 42 for the four teat cups. In the vacuum connection 31 there is incorporated a throttle ring 43 to prevent fluctuations in the vacuum of the various milk meters due to falling off of one of the teat cups. In order to be able to ascertain whether a vacuum is present in the milk meter, there is arranged an air flow sensor 44 in the vacuum connection between the throttle ring 43 and the relevant milk meter, which sensor supplies the computer 22 with a control signal S8 indicating the presence of vacuum in the line 31. Hereby this signal also forms an indication whether the teat cups are connected correctly.

Figure 4 shows a longitudinal cross-sectional view of a teat cup; this teat cup in a customary manner consists of a solid, e.g. metal, sleeve 45, an inner wall 46 made of a flexible material, e.g. rubber, enclosed thereby, and a rubber cap 47 which seals the space between the sleeve 45 and the inner wall 46 at the upper side. At the lower

side, the space between the sleeve 45 and the inner wall 46 is sealed by a sealing ring 48, while at some distance thereabove there is provided between the sleeve 45 and the inner wall 46 a ring 49 having an aperture 50. Between the sealing ring 48 and the ring 49 there is located a space, in which the electronic pulsator system 42 produces through the line 52 and via an aperture 51 a pulsating vacuum, thereby effecting in the space between the sleeve 45 and the inner wall 46 a pulsating vacuum which causes the inner wall 46 to close firmly around the teat, when the teat cup is connected thereto, or causes the inner wall to move outwards again, whereby is obtained the rhythmic movement around the teat as required for the milking operation to be performed. In order to function as a buffer for the milk to be collected and to minimize the fluctuations in the vacuum under the teat, there is provided in the lower part of the teat cup 2 a buffer space 53, in which a relatively narrow air suction aperture 54 is made for the milk transport. The line 3, intended for the discharge of the milk to the milk meter 7, is connected to this buffer space 53. In addition, the buffer space 53 contains a fixed element 55, which element partly projects into the aperture between the teat space and the buffer space 53 to ensure that the milk flows gradually into the buffer space 53 and a splitting of the milk is prevented. At the upper end of the fixed element 55 a sensor can be provided to perform a temperature measurement. The temperature of the milk indicates the body temperature of the cows to be milked; the latter temperature is higher than normally with cows in heat and with sick cows.

In order that the milk line system can be rinsed once the milking operation has been completed, it must include an arrangement for effecting this step. To that end, there is provided a rinse line system which is constituted by a rinse fluid container 56 having a valve 57, a first rinse line 58, a rinse jetter 59 which can be fitted around the end of the teat cup 2 in a fluid-tight manner, the teat cup 2, the line 3, the milk meter 7, the separate discharge line 8, the common discharge line 9, a three-way valve 60 incorporated therein and a second rinse line 61. In its first position, the three-way valve 60 admits milk from the common discharge line 9 into the circular line 5, and, in its second position, rinse fluid from the common line 9 into the second rinse line 61. After the milking, a rinse command can be delivered by the computer 22 which, to that end, applies a control signal S7 to the three-way valve 60 to adjust same to the appropriate position. Prior to starting the rinsing step after the milking operation has ended, the discharge lines must first be freed from milk. This is effected by passing compressed air through the measuring chamber 11, as

a result of which the spherical body 23 is pushed upwardly and the aperture 24 is released. In front of the three-way valve 60 there is incorporated in the common discharge line 9 an air-milk sensor 62, which sensor applies a control signal S9 to the computer, on the basis of which control signal the computer can establish when there is no longer any milk present in the common line - compressed air then passing the air-milk sensor 62 instead of milk - so that the valve 60 can be adjusted for the rinsing procedure. Due to the vacuum in the milk receptacle 10, the rinse fluid is sucked from the rinse fluid container 56 through the first rinse line 58, the rinse jetter 59, the teat cup 2 and the line 3 to the milk receptacle 10, from where it flows into the measuring chamber 11, whereafter it is pumped therefrom in the same manner as the milk and is fed back via the separate discharge line 8, the common discharge line 9, the three-way valve 60 and the second rinse line 61 to the rinse fluid container 56.

In the common discharge line 9 there is arranged in front of the three-way valve 60 incorporated therein a heat exchanger 63 as a pre-cooler for the milk cooling tank.

In customary manner, the milk cooling tank 6 is incorporated in the circular line 5, to which via relevant three-way valves 60 the common discharge lines of the individual milk meters 7 are connected. A pump 64 is incorporated in the circular line 5. This pump can operate at at least two different speeds. In order to keep the milk in motion and to prevent milk residues from being deposited in the circular line 5, the milk is circulated at a relatively low speed from the milk cooling tank 6 through the circular line 5. Preferably, in this connection, the circular line 5 is thermally insulated. Usually, the milk cooling tank 6 is emptied a few times a week, whereafter it can be rinsed. A rinsing fluid can be introduced into the milk cooling tank 6 via a valve 65 and a spray nozzle 66. When the spray nozzle 66 is arranged capably of moving, it can cover the entire inner surface of the milk cooling tank 6. The rinsing fluid is circulated by the pump in the circular line at a relatively high speed and is ultimately discharged via a three-way valve 67. Also here, the valves 65, 67, the spray nozzle 66 and the pump 64 can be controlled from the computer 22.

Although in Figure 2 they are shown as being single, several elements are provided in fourfold; in particular this holds for the rinse jetter, the teat cup and the milk meter, as well as for the lines connected thereto and the elements incorporated therein (valves and sensors). Preferably, the four milk meters are combined into one single milk measuring device.

Claims

1. A milking plant for milking cows, which plant comprises teat cups (2) that are connectable to the respective teats of a cow's udder, while the milk obtained from each udder quarter with the aid of the said teat cups (2) can be conveyed through a separate line (3) to a milk measuring device (4), characterized in that the milk measuring device (4) includes four milk meters (7), whose separate discharge lines (8) are coupled to a common discharge line (9) terminating in a milk cooling tank (6).
2. A milking plant as claimed in claim 1, characterized in that a milk meter (7) includes a milk receptacle (10) and a measuring chamber (11), in which connection the milk flows under a vacuum from the milk receptacle (10) into the measuring chamber (11) and is pumped in defined quantities by means of compressed air from the measuring chamber (11) into the relevant, separate discharge line (8).
3. A milking plant as claimed in claim 2, characterized in that the air sucked along by the vacuum together with the milk from the relevant teat cup (2) is separated therefrom in the milk meter (7).
4. A milking plant as claimed in claim 1 or 2, characterized in that the milk meter (7) is provided with a valve rod (12) which, in a first position under a vacuum, leaves the aperture (13) between the milk receptacle (10) and the measuring chamber (11) open, and, in a second position by means of compressed air, closes same.
5. A milking plant as claimed in claim 4, characterized in that there is provided a switching element (17), with the aid of which, in a first switching position thereof, the vacuum is applied to cause the valve rod (12) to be adjusted to its first position, and, in a second switching position thereof, compressed air is admitted to cause the valve rod (12) to be adjusted to its second position.
6. A milking plant as claimed in claim 5, characterized in that the measuring chamber (11) is provided with a milk level sensor (21), by means of which it is established when a defined quantity of milk is contained therein, after which it supplies a control signal (S1) to cause the switching element (17) to be adjusted from its first position to its second position.
7. A milking plant as claimed in claim 5 or 6, characterized in that the switching element (17) is adjusted from its second position to its first position after a fixed period of time.
8. A milking plant as claimed in claim 6, characterized in that, each time when a defined quantity of milk has been established in the measuring chamber (11), the milk level sensor (21) supplies a

control signal (S2) for a computer (22), in which the quantity of milk obtained from each udder quarter is recorded for each individual cow.

- 5 9. A milking plant as claimed in any one of claims 2 to 8, characterized in that the measuring chamber (11), when empty, is closed by a spherical body (23).
- 10 10. A milking plant as claimed in claim 9, characterized in that, when the milk flows from the milk receptacle (10) into the measuring chamber (11), the spherical body (23) floats on the milk contained therein.
- 15 11. A milking plant as claimed in claim 9 or 10, characterized in that the milk meter (7) is provided with a lifting magnet (26) for establishing a connection between the closed measuring chamber (11) and the discharge lines (8) by displacing the spherical body (23), when any residual milk must be removed by compressed air.
- 20 12. A milking plant as claimed in any one of the preceding claims, characterized in that each milk meter (7) of the milk measuring device (4) is provided with a milk conductivity sensor (27).
- 25 13. A milking plant as claimed in claim 12, characterized in that a separate discharge line (8) is provided with a three-way valve (28), through which the milk pumped from a relevant milk meter (7) is either discharged into the common discharge line (9) or, after it has been found that the milk originates from an inflamed quarter, into a receptacle (29) specially provided for the purpose.
- 30 14. A milking plant as claimed in claim 13, characterized in that the milk conductivity sensor (27) applies a control signal (S3) to a computer, in which it is determined whether or not the relevant milk originates from an inflamed quarter, after which, when the former appears to be the case, the computer (22) supplies a control signal (S5) to the three-way valve (28) in the relevant separate discharge line (8) in order that the milk in question is discharged to the receptacle (29).
- 35 15. A milking plant as claimed in any one of the preceding claims, characterized in that a milk meter (7) is provided with a tube stop valve (30), with the aid of which the vacuum connection is disconnected from the milk meter (7) after milking.
- 40 16. A milking plant as claimed in claim 15, characterized in that, after a preset milking period laid down in a computer (22) has ended, the tube stop valve (30) is activated by a control signal (S6) supplied by the computer (22).
- 45 17. A milking plant as claimed in claim 15 or 16, characterized in that the tube stop valve (30) includes a lifting mechanism (36) to admit air into the milk meter (7) simultaneously with or immediately after closure of the vacuum connection of the milk meter (7).
- 50 18. A milking plant as claimed in any one of

the preceding claims, which plant includes a vacuum pump (38) and a vacuum balance tank (39) to increase the vacuum stability, characterized in that the vacuum connection from the balance tank (39) to a milk meter (7) includes a throttle ring (43).

19. A milking plant as claimed in claim 18, characterized in that, in order to ascertain whether a vacuum is present, the vacuum connection between the throttle ring (43) and the milk meter (7) is provided with an air flow sensor (44).

20. A milking plant as claimed in any one of the preceding claims, characterized in that at the lower end of a teat cup (2) there is provided a buffer space (53) for the milk transport and for counteracting fluctuations in the vacuum under the teat to which the teat cup (2) is connected.

21. A milking plant as claimed in claim 20, characterized in that the buffer space (53) contains a fixed element (55), which partly projects into the aperture between the teat space of the teat cup (2) and the buffer space (53) to ensure that the milk flows gradually into the buffer space (53).

22. A milking plant as claimed in claim 20 or 21, characterized in that a teat cup (2) is provided with a sensor to establish the milk temperature.

23. A milking plant as claimed in any one of the preceding claims, characterized in that the common discharge line (9) is provided with a three-way valve (60), through which in a first position thereof the milk is passed via the common discharge line (9) to the milk cooling tank (6), while in its second position the three-way valve (60) establishes a rinse line system.

24. A milking plant as claimed in claim 23, characterized in that the rinse line system includes a rinse jetter (59) which, for the purpose of flushing the lines, is connected to a teat cup (2), while a rinse fluid is passed by the vacuum in a milk meter (7) from a rinse fluid container (56) through a first rinse line (58) to the rinse jetter (59) and from there, via a teat cup (2), into the relevant milk meter (7), whereafter the rinse fluid is returned through the relevant separate discharge line (8), the common discharge line (9), the three-way valve (60) incorporated therein, and a second rinse line (61) to the rinse fluid container (56).

25. A milking plant as claimed in any one of the preceding claims, characterized in that the milk cooling tank (6) is included in a circular line (5), to which the common discharge lines (9) of the individual milk meters (7) are connected.

26. A milking plant as claimed in claim 25, characterized in that the circular line (5) is provided with a pump (64) for circulating the milk from the milk cooling tank (6) therethrough.

27. A milking plant for milking cows, which plant includes a milk cooling tank (6), characterized in that the milk cooling tank (6) is included in a

circular line (5), to which the discharge lines (9) of the individual milk meters (7) are connected.

28. A milking plant as claimed in claim 26 or 27, characterized in that the milk cooling tank (6) is provided with a rinsing fluid supply line, through which, after all the milk has been removed from the milk cooling tank (6), a rinsing fluid can be admitted into the milk cooling tank (6), which rinsing fluid is circulated through the circular line (5) by means of the pump (64).

29. A milking plant as claimed in claim 28, characterized in that the pump (64) can operate at two speeds, the milk being circulated at a relatively low speed and the rinsing fluid at a relatively high speed.

30. A milking plant as claimed in claim 28 or 29, characterized in that a three-way valve (67) is incorporated in the circular line (5), by means of which the circulating rinsing fluid can be discharged.

25

30

35

40

45

50

55

FIG. 1

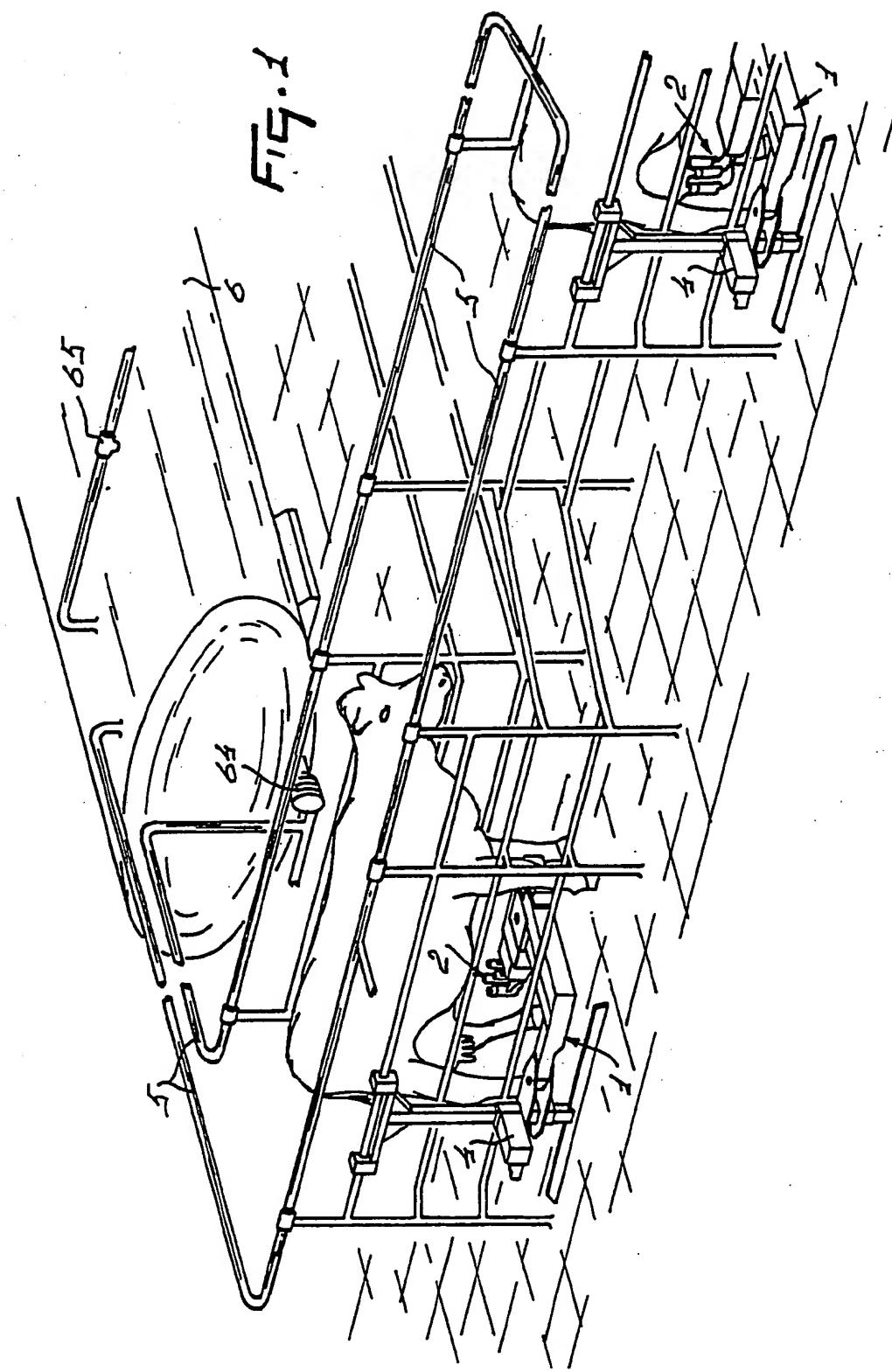
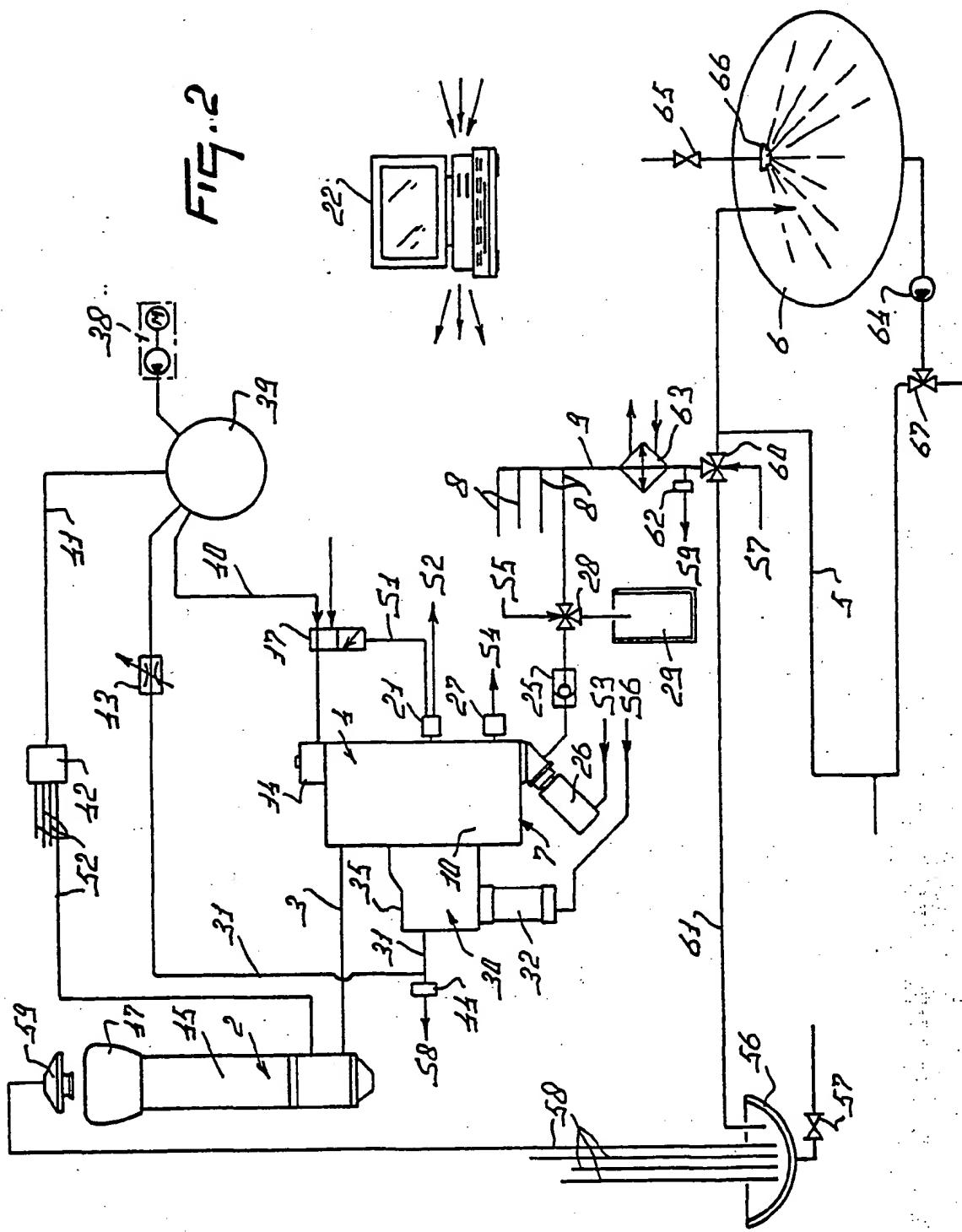


FIG. 2



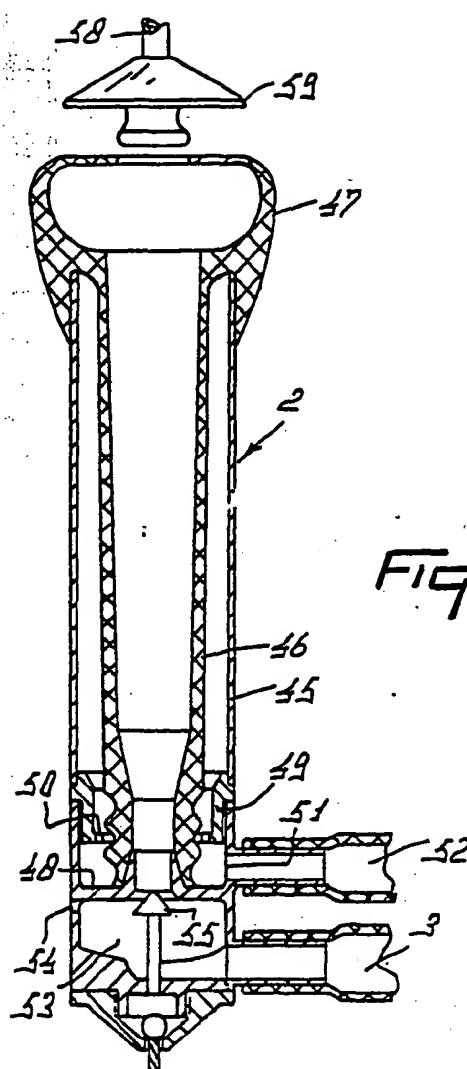


FIG. 4

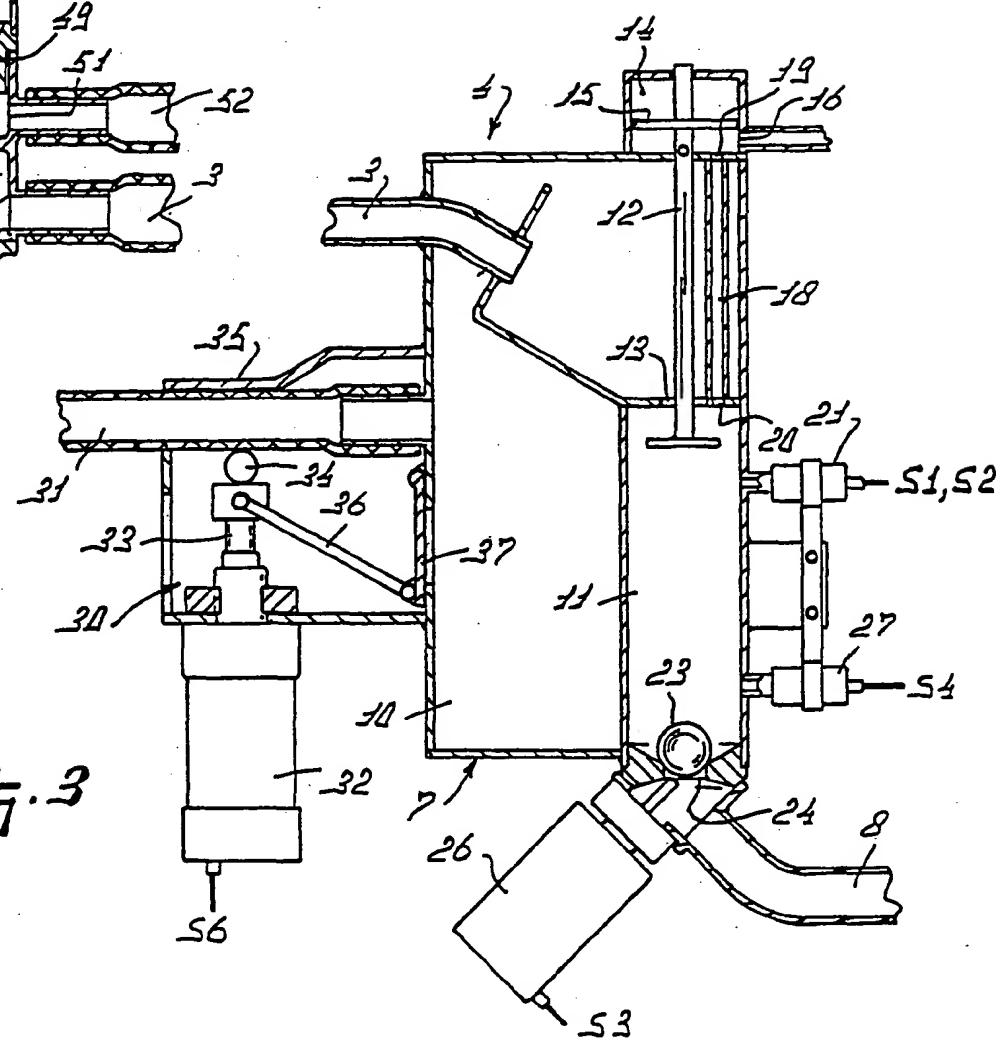


FIG. 3

Beställare

PATMTH patmth

Ärende

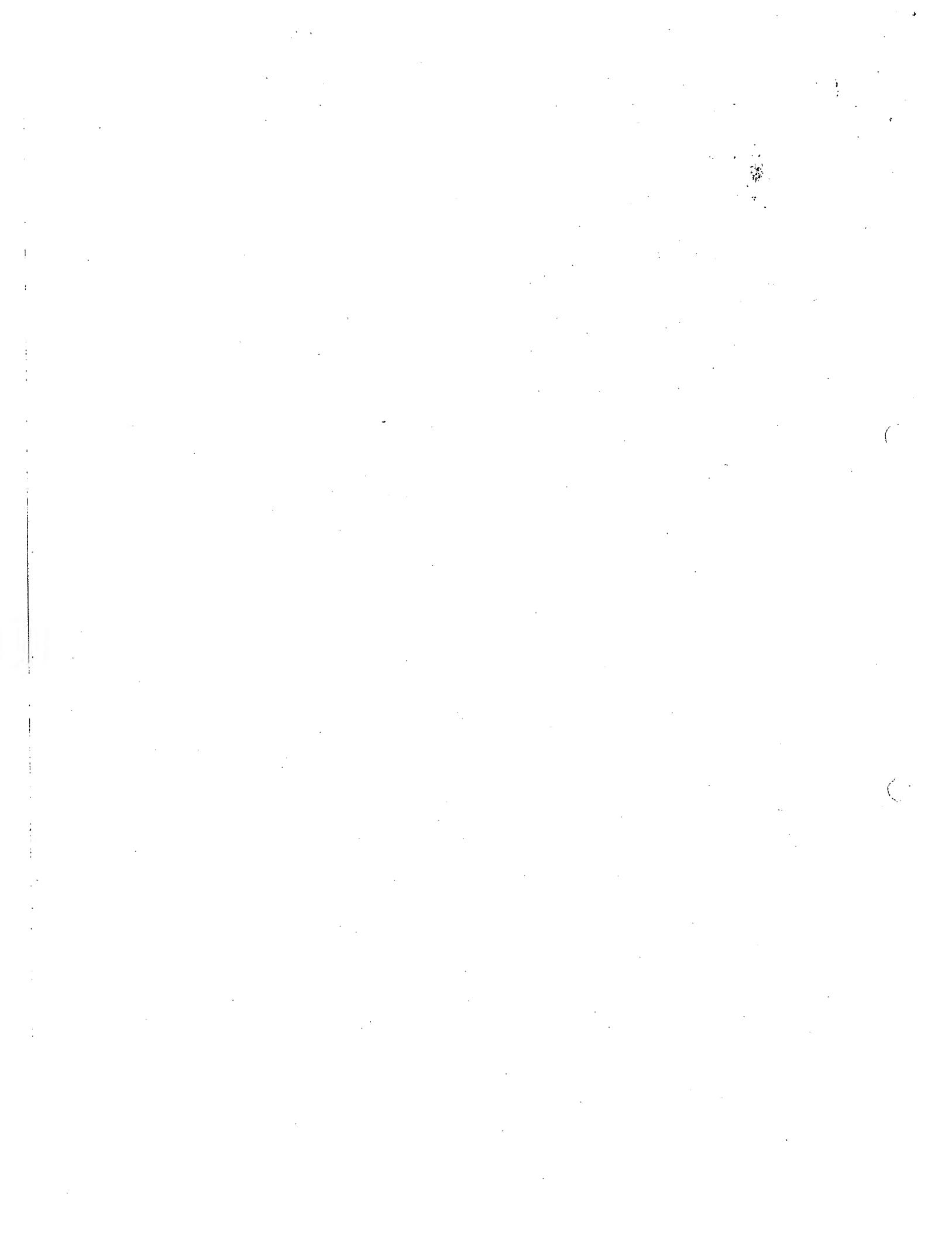
epd90606

Dokument

EP 0397583A1 I

Antal sidor utöver denna

9





Europäisches Patentamt
European Patent Office
Office européen des brevets

⑪ Numéro de publication:

0 397 583
A1

⑫

DEMANDE DE BREVET EUROPEEN

㉑ Numéro de dépôt: 90420212.4

㉑ Int. Cl.⁵: C12Q 1/04, G01N 33/50,
G01N 33/04, G01N 1/30

㉒ Date de dépôt: 02.05.90

㉓ Priorité: 12.05.89 FR 8906525

㉑ Demandeur: L.E.C.A. (LABORATOIRE
D'ETUDES EN CYTOMETRIE APPLIQUEE)
20 cours Becquart Castelbon
F-38500 Voiron(FR)

㉔ Date de publication de la demande:
14.11.90 Bulletin 90/46

㉒ Inventeur: Batandier, Cécile
4 rue Charles Testoud
F-38000 Grenoble(FR)
Inventeur: Perrot, Guy
La Murette
F-38140 Rives(FR)

㉕ Etats contractants désignés:
BE DE FR GB NL

㉔ Mandataire: Laurent, Michel et al
Cabinet Laurent et Charras 20, rue Louis
Chirpaz B.P. 32
F-69131 Ecully Cédex(FR)

㉖ Procédé et dispositif d'identification et de numérisation de cellules dans du lait.

㉗ Procédé pour identifier et numériser les cellules contenues dans du lait caractérisé en ce qu'il consiste:

- à prélever une quantité déterminée d'un échantillon d'un lait à analyser ;
- à colorer les cellules présentes dans cet échantillon par un colorant fluorescent spécifique de l'ADN contenu dans le noyau desdites cellules ;
- à faire défiler cet échantillon coloré dans un tube capillaire (13) sur lequel est dirigé un faisceau lumineux stabilisé ;

A1
11 583 A1
EP 0 397 583 A1
- à recueillir sur un capteur une partie au moins du rayon lumineux transmis, donnant une première information, représentative du nombre de cellules qui défilent ;

- à traiter cette information ;
- et enfin, à restituer au moins une valeur représentative de la qualité du lait analysé.

Application : diagnostic de la mammité chronique chez les vaches.

PROCEDE ET DISPOSITIF D'IDENTIFICATION ET DE NUMERISATION DE CELLULES DANS DU LAIT.

La présente invention concerne un procédé pour détecter et numériser les cellules présentes dans du lait et notamment dans du lait de vache. Elle concerne également le dispositif mettant en œuvre ce procédé.

Les produits alimentaires d'origine animale sont soumis à un contrôle vétérinaire qui, au fur et à mesure des progrès de la recherche scientifique, tendent à s'intensifier, afin de proposer au consommateur des produits de qualité.

Les produits laitiers sont particulièrement soumis à ces contrôles, du fait de leur propension à rapidement s'altérer. Parmi ceux-ci, le lait de vache, très largement consommé, subit des examens vétérinaires systématiques, notamment en vue de révéler la présence en nombre relativement important de cellules, d'origine épithéliale en phase de désquamation et en voie de lyse, mais également d'origine inflammatoire, tels que des lymphocytes et autres leucocytes, révélatrices de pathologie de la vache concernée, et notamment de mammites chroniques.

Cette infection, bien que sans grande incidence sur l'état général de la vache, rend le lait tout à fait impropre à la consommation. De fait, un dépistage systématique de cette infection est réalisé sur le lait. Ce dépistage peut être effectué à différents niveaux, soit par le producteur dès que la traite est effectuée, soit par des services vétérinaires, soit par les laiteries ou les industries de transformation.

A ce jour, on utilise deux types de techniques. La première consiste en une appréciation approximative du nombre de cellules contenues dans un échantillon, à l'aide d'un détergent, du type connu sous la marque déposée TEEPOL, ou d'un indicateur colorimétrique. La deuxième technique, utilisée par les professionnels, consiste en un comptage des cellules soit de façon manuelle, impliquant la présence de personnel spécialisé, soit de façon automatisée au moyen d'un matériel approprié, qui ne peut hélas différencier les cellules entières des débris cellulaires, et qui de plus demande un étaffonnage manuel préalable sur au moins deux échantillons.

Parmi ces différentes méthodes, HAGELTORN et al ont proposé (voir BIOLOGICAL ABSTRACTS, vol. 82, n° 12, 1986, page AB-458, & AM. J. VET. RES., 47(9), pages 2012-2016, 1986) un procédé apte à identifier des cellules contenant dans de l'ADN ou acide désoxyribonucléique, que de l'ARN ou acide ribonucléique, marquées préalablement de manière spécifique au moyen d'un colorant supravital, l'Acridine Orange. Cette identification est réalisée grâce à une localisation géographique des différentes populations cellulaires re-

pertoriées sur un diagramme de répartition, obtenu après analyse en cytométrie de la taille et de la densité desdites cellules. Ce procédé vise plus spécifiquement à déterminer les excès de ces diverses populations cellulaires par rapport à des données standard, répertoriées dans des banques de données, qu'à réaliser un comptage cellulaire des échantillons étudiés. Ces excès obtenus de manière relative, ne permettent pas d'aboutir à des valeurs absolues, qui seules sont réellement représentatives de la qualité du lait analysé. En outre, pour le colorant spécifique utilisé, la coloration stoechiométriques sur l'ADN et sur l'ARN n'est obtenue que pour des conditions opératoires strictes, donc incompatibles avec une technique automatisée. Il est donc inutilisable dans le cadre d'analyse pour lesquelles la totalité des cellules, tant mortes que vivantes doivent être prises en compte, et notamment, dans le cas du dépistage systématique des mammites chroniques. Enfin, comme déjà dit, ce procédé reposant sur la comparaison avec des données de base, préalablement intégrées, suppose une connaissance approfondie du domaine considéré, et demande donc un personnel qualifié pour sa mise en œuvre.

On a également proposé dans le brevet US-A-3679365 une méthode de comptage des cellules somatiques dans le lait, consistant à colorer préalablement la totalité de l'échantillon de lait à analyser. De la sorte, et afin de pouvoir réaliser le comptage ultérieur, une étape supplémentaire dite de "clarification", destinée à faire éclater les globules lipidiques au moyen d'un agent tensio-actif est rendue nécessaire, accroissant de fait la durée des analyses.

On a également proposé une méthode plus spécifiquement destinée à l'identification de bactéries, et reposant sur le principe de la fluorométrie. Cette méthode, décrite par exemple par SHELLY et al dans CLINICAL CHEMISTRY, vol.29, n° 2, 1983, pages 290-296, consiste à faire subir à un échantillon une double coloration, à l'origine d'une double fluorescence, dont le rapport relatif mesuré et analysé permet de remonter au type de bactéries. Ce procédé suppose donc la connaissance préalable des rapports relatifs de fluorescence de chaque type de bactérie, et donc corollairement, ne peut être employé que par du personnel qualifié. En outre, si certes, l'identification des bactéries est obtenue de manière relativement précise, en revanche, cette méthode s'avère impropre pour la numérisation cellulaire, et donc pour le dépistage des mammites chroniques, objet principal de l'invention.

La présente invention vise à pallier ces incon-

3 vénients. Elle vise un procédé et un dispositif apte à permettre un diagnostic infraclinique de la mammite chronique, qui soit sûr et sans app I, par voie automatique, précis, rapide et de coût de fonctionnement léger.

Ce procédé pour identifier et numériser les cellules dans du lait est caractérisé en ce qu'il consiste:

- à prélever un échantillon d'un lait à analyser ;
- à colorer les cellules présentes dans cet échantillon par un colorant fluorescent spécifique de l'ADN (Acide desoxyribonucléique) contenu dans le noyau des cellules ;
- à faire défiler cet échantillon coloré dans un tube capillaire sur lequel est dirigé un faisceau laser stabilisé ;
- à recueillir sur un capteur une partie au moins du rayon lumineux transmis à travers le flux de l'échantillon s'écoulant dans le tube capillaire pour donner une première information I_1 , significative de la quantité d'ADN, donc du nombre de cellules qui défilent ;
- à transmettre ladite information I_1 à une unité de traitement ;
- et enfin, à restituer au moins une valeur représentative de la qualité du lait analysé.

En d'autres termes, la présente invention consiste à analyser par cytofluorométrie un échantillon de lait préalablement coloré par un colorant approprié, afin de déterminer notamment la quantité et la qualité des cellules contenues dans ledit échantillon de lait, et à traiter les informations ainsi recueillies pour déterminer la qualité de lait contenue dans ledit échantillon.

Avantageusement, en pratique :

- lors de la phase de coloration, on ajoute en outre un autre colorant fluorescent, apte à induire une réaction enzymatique spécifique d'une famille des constituants contenus dans lesdites cellules, puis on recueille sur un capteur, partie du rayon lumineux transmis à travers le flux de l'échantillon s'écoulant dans le tube capillaire pour donner une information, représentative de la quantité de la dite famille de constituants cellulaire ;
- simultanément, on recueille sur des capteurs, respectivement :
 - . une partie du rayon lumineux diffracté à 90° de l'axe optique défini par le faisceau lumineux initial pour donner une seconde information I_2 , significative de la densité desdites cellules ;
 - . au moins une partie du rayon lumineux légèrement diffracté au voisinage de l'axe optique, pour donner une troisième information I_3 , significative de la taille desdites cellules ;
- les dites informations I_2 et I_3 étant alors transmises à la dite unité de traitement puis restituées sous forme de valeur(s) représentative(s) de la qualité du lait analysé ;

5 - simultanément à la coloration, on ajoute au lait un milieu hypotonique, destiné à lyser la membrane cellulaire, et un détergent tensio-actif destiné à perméabiliser la membrane nucléaire afin de permettre la pénétration du colorant fluorescent destiné à venir se fixer sur l'ADN ;

10 - l'agent tensio-actif est introduit dans le milieu à raison de 0,1 % en volume, et l'agent hypotonique est du citrate de sodium à raison de 0,1 % en rapport Poids/Volume ;

15 - le colorant fluorescent spécifique de l'ADN est de l'iode de propidium, introduit en excès par rapport à la quantité d'ADN ;

- on laisse incuber le milieu réactionnel à température ambiante et dans l'obscurité pendant au moins vingt minutes ;

20 - on fait défiler le milieu réactionnel au moyen d'un venturi à pression différentielle.

L'invention concerne également un dispositif pour la mise en oeuvre du procédé comprenant :

- un rayon lumineux stabilisé ;
- une unité d'analyse constituée par :
 - . une cellule de lecture réalisée en quartz, située sur le trajet optique du faisceau laser ;
 - . un système à pression différentielle destiné à permettre le passage dans ladite cellule de lecture de l'échantillon de lait à analyser ;
 - . un réservoir destiné à recueillir l'échantillon après analyse ;

25 - au moins trois capteurs optiques dont deux sont munis d'un photomultiplicateur ;

- un microprocesseur apte à assurer le traitement des informations issues dudit capteur ;

- un organe d'affichage et/ou d'impression.

30 Avantageusement, en pratique :

- le rayon lumineux stabilisé est un rayon laser ;

- les trois capteurs sont constitués par des photodiodes ;

35 - le système à pression différentielle est constitué par un venturi ;

- l'échantillon à analyser s'écoule dans un capillaire au sein de la cellule d'analyse.

40 La manière dont l'invention peut être réalisée et les avantages qui en découlent ressortiront mieux de l'exemple de réalisation qui suit, donné à titre indicatif et non limitatif à l'appui des figures annexées, dans lesquelles :

45 La figure 1 représente une vue schématique du système d'écoulement de l'échantillon en vue de son analyse du dispositif conforme à l'invention.

50 Les figures 2 et 3 représentent des courbes établissant le nombre d'entités, à savoir débris cellulaires et cellules entières, en fonction de la quantité d'ADN mesurée.

55 Conformément à la présente invention, l'échantillon de lait devant être analysé est tout d'abord soumis à un traitement préalable. En effet, on laisse incuber une quantité exactement mesurée de

l'échantillon, et ce environ une vingtaine de minutes dans un milieu hypotonique, tel que du citrate de sodium 0,1% en présence d'un agent tensio-actif (telle que du Triton (Marque Déposée)). La concentration de ce dernier est de l'ordre de 0,1 % en volume. On inclut également dans ce mélange réactionnel un colorant fluorescent dont la caractéristique essentielle est de se fixer spécifiquement sur l'Acide Desoxyribo Nucléique (ADN) contenu dans le noyau des cellules et ce, de manière stoechiométrique. On choisit pour cela de l'iodure de propidium connu pour cette application. Afin d'être sûr que la totalité de l'ADN soit coloré, on ajoute l'iodure de propidium en excès.

Afin de ne pas exciter les molécules d'iodure de propidium, on réalise cette incubation à l'abri de la lumière.

L'agent tensio-actif permet une lésion des membranes cellulaires permettant une pénétration de l'iodure de propidium au niveau de l'ADN nucléaire.

De manière connue, toutes les cellules non tumorales d'une même espèce contiennent la même quantité d'ADN. De la sorte, la quantité de fluorescence mesurée ultérieurement constitue une donnée absolue quant à la quantité de cellules analysées.

Afin d'assurer une mesure analytique de l'échantillon de lait à analyser, on a recours à un système de venturi à pression différentielle en liaison avec un capillaire permettant la création d'un courant laminaire de dimensions très réduites dudit échantillon, destiné à être placé, sous la forme d'un flux monocellulaire, sur le chemin optique du dispositif de mesure décrit ultérieurement. Pour ce faire, on fait appel au dispositif représenté au sein de la figure 1. Ce dispositif comprend une unité d'analyse dont l'élément essentiel est l'organe d'écoulement de l'échantillon. Ce dernier comprend fondamentalement une cellule de lecture réalisée en quartz apte à permettre la transmission d'un faisceau lumineux, notamment d'un faisceau laser. Les parois de cette cellule de lecture sont avantageusement planes et parallèles, afin de diminuer les éventuels phénomènes de réflexion partielle ou totale.

Une pompe à air (5), comprimant l'air extérieur préalablement filtré (6), et munie d'un régulateur de pression (7) permet d'envoyer le fluide porteur depuis son réservoir (8) jusqu'à la cellule de lecture (3), le dit fluide porteur, en l'occurrence de l'eau permutée étant également filtré (9), notamment avec un filtre stérilisant, avant son introduction dans la chambre (12) du venturi. En parallèle, une boucle de contrôle fermée (10), régule la pression différentielle permettant à l'échantillon de lait (2) de passer par l'intermédiaire d'un tube capillaire (4) dans la cellule de lecture (3). Les parois de la

chambre (12) du venturi convergent au niveau de la dite cellule de lecture (3), afin de former à ce niveau un capillaire (13). C'est dans ce capillaire (13) qu'est concentré le spot du faisceau lumineux utilisé pour le système de mesure et décrit ultérieurement.

Pour compléter l'unité de traitement (1) ainsi définie, on munit l'ensemble d'un réservoir (11) recevant l'échantillon et le fluide support après analyse.

Le système de mesure conforme à l'invention comprend essentiellement un laser à Argon de 15 milliwatts, produisant un faisceau stabilisé de longueur d'onde bien déterminée égale à 488 nm. Le faisceau Laser ainsi produit est soigneusement collimaté par tout organe approprié et focalisé au niveau du capillaire (13) de la cellule de lecture (3). La longueur d'onde du faisceau laser produit tombe avantageusement au voisinage du maximum d'absorption de l'iodure de propidium, utilisé pour colorer l'ADN cellulaire. Lorsque ce dernier est excité, il réemet une radiation dite de fluorescence de longueur d'onde bien connue et égale à 620 nm. Cette radiation de fluorescence est détectée au moyen d'un capteur constitué essentiellement d'un photomultiplicateur, mesurant l'intensité du radiation émise selon un signal I_1 , et de fait, la quantité d'ADN s'étant écoulé devant le faisceau laser. Cette dernière grandeur permet de remonter au nombre de cellules ayant transité par le capillaire (13), car comme déjà dit, la quantité d'ADN cellulaire est une constante constituant de la sorte un quantum de repère. Il est à noter qu'il existe toutefois un petit nombre de cellules en phase de mitose. De fait, leur quantité d'ADN est double par rapport aux cellules en Interphase.

Il va de soi que pour parvenir à une précision suffisante, le capillaire (13) doit avoir des dimensions suffisamment réduites afin de n'autoriser l'écoulement simultané que d'un nombre très limité de cellules, pour que la totalité des cellules émette une radiation de fluorescence via l'iodure de propidium.

L'information I_1 ainsi obtenue est en elle-même nécessaire et suffisante pour estimer la qualité du lait. Toutefois, afin de disposer d'éléments supplémentaires d'appréciation, on procède à d'autres mesures. Ainsi, une partie du faisceau laser est diffractée par les cellules selon une grande variété d'angles par rapport à l'axe optique défini par le faisceau lui-même. On place de fait à 90 degrés par rapport à cet axe optique un second capteur, constitué par une photodiode, afin de délivrer un signal I_2 utilisable. Ce signal I_2 est représentatif de la densité des cellules s'écoulant dans le capillaire (13).

On place aussi à environ 10 degrés de l'axe optique défini précédemment un troisième capteur,

également constitué par un photomultiplicateur, et détectant une partie du faisceau laser diffracté par les cellules. Les informations détectées à ce niveau correspondent à une partie du faisceau laser ayant été diffractée lors de la traversée des cellules par ledit faisceau. De la sorte, l'information I_3 que ce capteur restitue est représentatif de la taille des dites cellules.

De la sorte, on dispose d'au moins trois informations I_1 , I_2 et I_3 correspondant respectivement au nombre, à la taille et à la densité des cellules s'écoulant dans le capillaire (13).

Ces informations sont traitées au moyen d'un microprocesseur susceptible d'effectuer une sélection parmi les informations transmises, en fonction de paramètres introduits dans le dit microprocesseur par l'utilisateur.

Il est par exemple nécessaire dans la détermination du nombre de cellules par unité de volume de lait, de s'affranchir des cellules non entières. Il suffit dans ce cas de fixer un seuil pour la fluorescence, en dessous duquel les entités détectées ne sont pas comptabilisées comme des cellules, mais comme des débris, d'origine cellulaire. Ce seuil est très facilement déterminé. Il correspond en effet, à la quantité de fluorescence équivalente, dans le cas du lait, donc des mammifères à la quantité d'ADN contenue dans le stock diploïde des $2n$ chromosomes. De fait, toutes les entités, dont l'intensité de fluorescence dépasse ce seuil sont des cellules entières. Dans les figures 2 et 3, on a représenté par S_1 ce seuil. On observe sur ces courbes, un pic très marqué, correspondant aux cellules entières en interfase. La détermination de la quantité d'ADN au dessus de ce seuil permet donc d'obtenir avec précision le nombre de cellules entières, aux cellules en cours de mitose près.

Comme déjà dit, toutes les entités dont le signal de fluorescence apparaît en dessous du seuil S_1 sont des débris cellulaires.

De manière avantageuse, le rapport des débris cellulaires sur la quantité d'ADN totale et le rapport des cellules entières sur la quantité d'ADN totale sont déterminés, afin de donner avec une plus grande précision l'état pathologique de l'animal concerné.

De même, la différenciation entre particules cellulaires et non cellulaires (par exemple globules lipidiques, très nombreux dans le cas du lait) s'effectue, dans le cas de mesure de fluorescence directe, par la sélection d'une plage d'intensité correspondant à la quantité connue d'ADN cellulaire.

De la sorte, on obtient une valeur représentative de la qualité du lait analysé, sous la forme d'un nombre de cellules par unité de volume, voire d'un graphique matérialisant les différentes informations mesurées, tels que ceux représentés au sein des

figures 2 et 3, et ce au moyen de tout organe de restitution sensorielle appropriée, tel que par exemple un écran et/ou un imprimante.

L'utilisateur est donc à même de déterminer rapidement la qualité de son lait. De fait, les litiges potentiels entre producteurs et laiteries, ou entre laiteries et consommateurs peuvent être largement diminués, la résultante générale conduisant à l'amélioration de la qualité du lait.

Dans une mise en œuvre avantageuse de l'invention, il est possible de procéder à une coloration multiple, en faisant appel à des colorants bien spécifiques, et ce, en vue d'obtenir la population de ou des différents types cellulaires contenus dans le lait, à savoir, par exemple, les polynucléaires, les lymphocytes, les macrophages ou monocytes et les cellules épithéliales.

Pour ce faire, on a recours à une réaction enzymatique propre à chacune desdites espèces. En d'autres termes, on fait réagir, lors de l'étape de coloration, un substrat d'une enzyme spécifique contenue dans l'espèce dont on désire mesurer la population.

Par exemple, lorsque l'on désire connaître, outre la population totale en cellules d'un échantillon de lait, la population particulière en polynucléaires, on adjoint à l'iode de propidium du phosphate de méthyl fluorescéine, ce dernier réagissant spécifiquement avec lesdits polynucléaires.

Le processus de l'analyse est alors rigoureusement le même que celui précédemment décrit, à savoir phase d'excitation à 488 nm, seule la phase de lecture s'effectuant à une longueur d'onde de 520 nm.

De la sorte, les résultats obtenus permettent de préciser la quantité relative de polynucléaires, donnée particulièrement intéressante pour établir un diagnostic, notamment, lorsque le seuil fixé à 500 000 cellules par millilitre n'est pas atteint.

Ainsi, le procédé conforme à l'invention permet-il d'une part une numérisation des différents constituants cellulaires du lait par des colorants spécifiques tel l'iode de propidium et tous ses équivalents, et d'autre part, une caractérisation des cellules comptées au moyen d'un marquage enzymatique spécifique d'un type cellulaire avec des substrats fluorescents.

Enfin, l'utilisation du cadrage des plages cellulaires présentant des différences de taille et/ou de nature permet d'améliorer sensiblement la précision du diagnostic.

Revendications

1/ Procédé pour identifier et numériser les cellules contenues dans du lait caractérisé en ce qu'il consiste :

- à prélever une quantité déterminée d'un échantillon d'un lait à analyser ;
- à colorer les cellules présentes dans cet échantillon par un colorant fluorescent spécifique de l'ADN (Acide desoxyribonucléique) contenu dans le noyau desdites cellules ;
- à faire défiler cet échantillon coloré dans un tube capillaire (13) sur lequel est dirigé un faisceau lumineux stabilisé ;
- à recueillir sur un capteur une partie au moins du rayon lumineux transmis à travers le flux de l'échantillon s'écoulant dans le tube capillaire (13) pour donner une première information I_1 significative de la quantité d'ADN, donc du nombre de cellules qui défilent ;
- à transmettre ladite information I_1 à une unité de traitement ;
- et enfin, à restituer au moins une valeur représentative de la qualité du lait analysé.

2/ Procédé selon la revendication 1, caractérisé en ce que, lors de la phase de coloration, on ajoute en outre un autre colorant fluorescent, apte à induire une réaction enzymatique spécifique d'une famille des constituants contenus dans lesdites cellules ;

- et, en ce que, l'on recueille sur un capteur, partie du rayon lumineux transmis à travers le flux de l'échantillon s'écoulant dans le tube capillaire (13) pour donner une information, représentative de la quantité de la dite famille de constituants cellulaires

3/ Procédé selon la revendication 1, caractérisé en ce que, simultanément, on recueille sur des capteurs, respectivement :

- une partie du rayon lumineux diffracté à 90° de l'axe optique défini par le faisceau lumineux initial pour donner une seconde information I_2 , significative de la densité desdites cellules ;
 - au moins une partie du rayon lumineux légèrement diffracté au voisinage de l'axe optique, pour donner une troisième information I_3 , significative de la taille desdites cellules ;
- les dites informations I_2 et I_3 étant alors transmises à la dite unité de traitement puis restituées sous forme de valeur(s) représentative(s) de la qualité du lait analysé.

4/ Procédé selon l'une des revendications 1 à 3, caractérisé en ce que simultanément à la coloration, on ajoute au lait un milieu hypotonique, destiné à lyser la membrane cellulaire, et un détergent tensio-actif destiné à perméabiliser la membrane nucléaire afin de permettre la pénétration du colorant fluorescent destiné à venir se fixer sur l'ADN.

5/ Procédé selon la revendication 4, caractérisé en ce que l'agent tensio-actif est introduit dans le milieu à raison de 0,1 % en volume, et l'agent

hypotonique est du citrate de sodium à raison de 0,1 % en rapport Poids/Volume.

6/ Procédé selon l'une des revendications 1 à 5, caractérisé en ce que le colorant fluorescent spécifique de l'ADN est de l'iодure de propidium, introduit en excès par rapport à la quantité d'ADN.

7/ Procédé selon l'une des revendications 1 à 6, caractérisé en ce qu'on laisse incuber le milieu réactionnel à température ambiante et à l'abri de la lumière pendant au moins vingt minutes.

8/ Procédé selon l'une des revendications 1 à 7, caractérisé en ce qu'on fait écouler le milieu réactionnel au moyen d'un venturi (5,7,8,10,12) à pression différentielle.

9/ Procédé selon l'une des revendications 1 à 8, caractérisé en ce que le faisceau lumineux stabilisé est un faisceau laser.

10/ Dispositif pour la mise en œuvre du procédé selon l'une des revendications 1 à 9, caractérisé en ce qu'il comprend :

- un laser stabilisé ;
- une unité d'analyse (1) constituée par :
 - . une cellule de lecture (3) réalisée en quartz, située sur le trajet optique du faisceau laser ;
 - . un système à pression différentielle destiné à permettre le passage dans ladite cellule de lecture (3) de l'échantillon de lait à analyser (2) ;
 - . un réservoir (11) destiné à recueillir l'échantillon après analyse ;
- un laser stabilisé ;
- trois capteurs optiques, dont deux sont munis d'un photomultiplicateur ;
- un microprocesseur apte à assurer le traitement des informations issues dudit capteur ;
- un organe d'affichage et/ou d'impressions.

11/ Dispositif selon la revendication 10, caractérisé :

- en ce que les capteurs sont constitués par des photodiodes ;
- en ce que l'échantillon à analyser (2) s'écoule dans un capillaire (13) au sein de la cellule de lecture (3) ;
- et, en ce que le système à pression différentielle est constitué par un venturi.

45

50

55

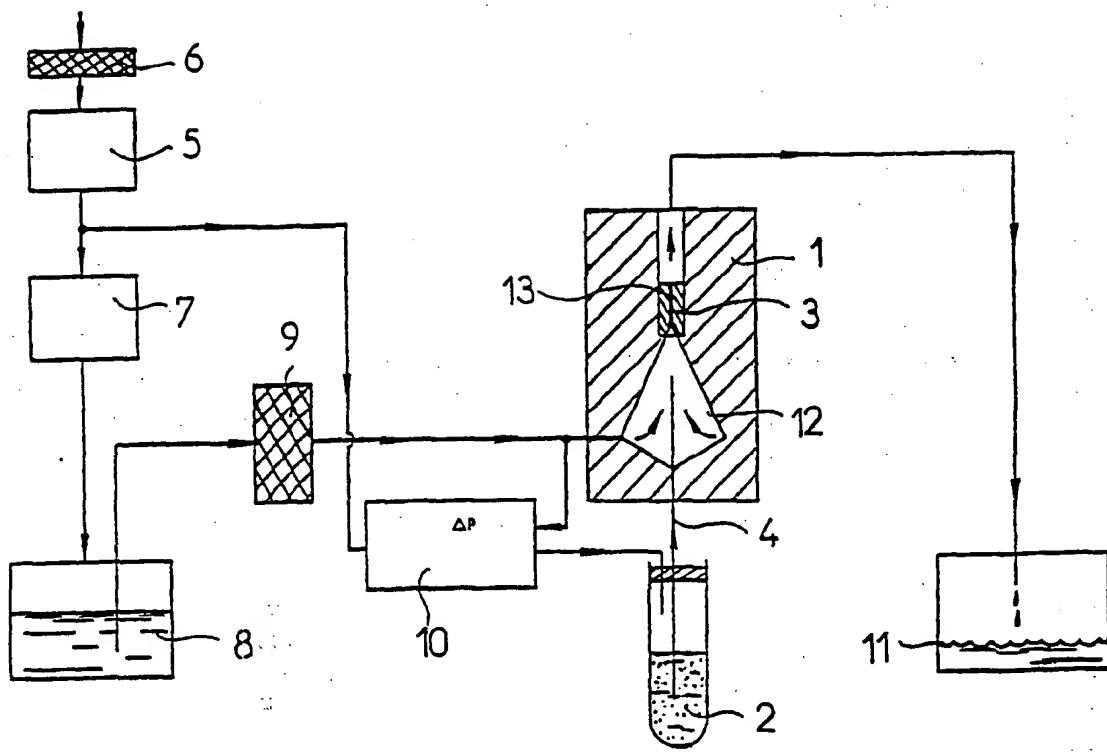


FIG 1

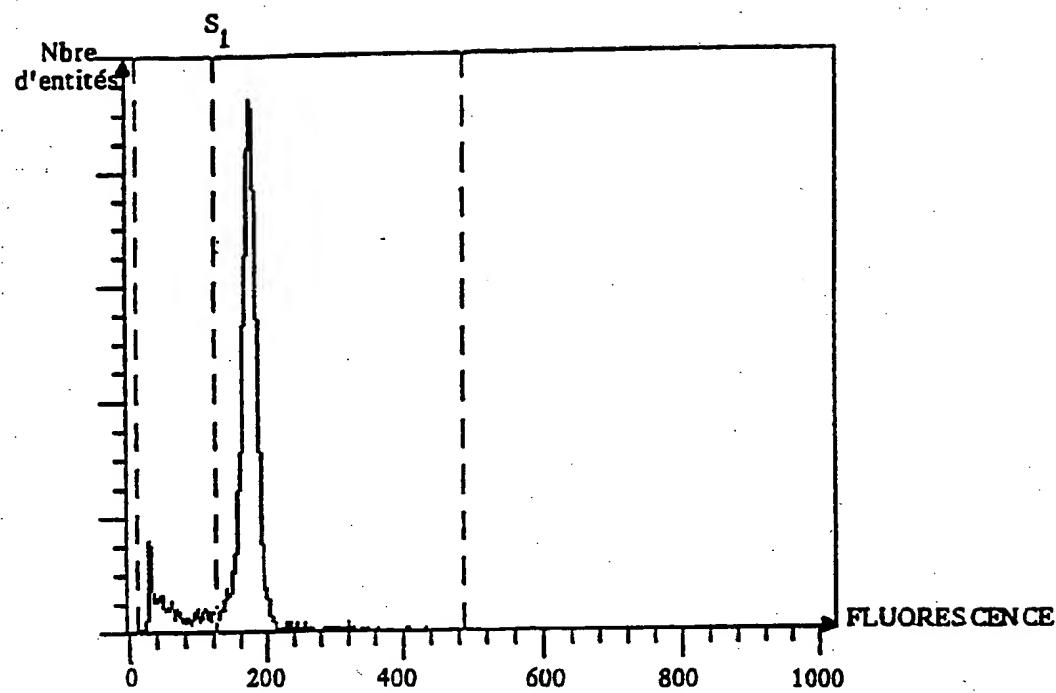


FIG 2

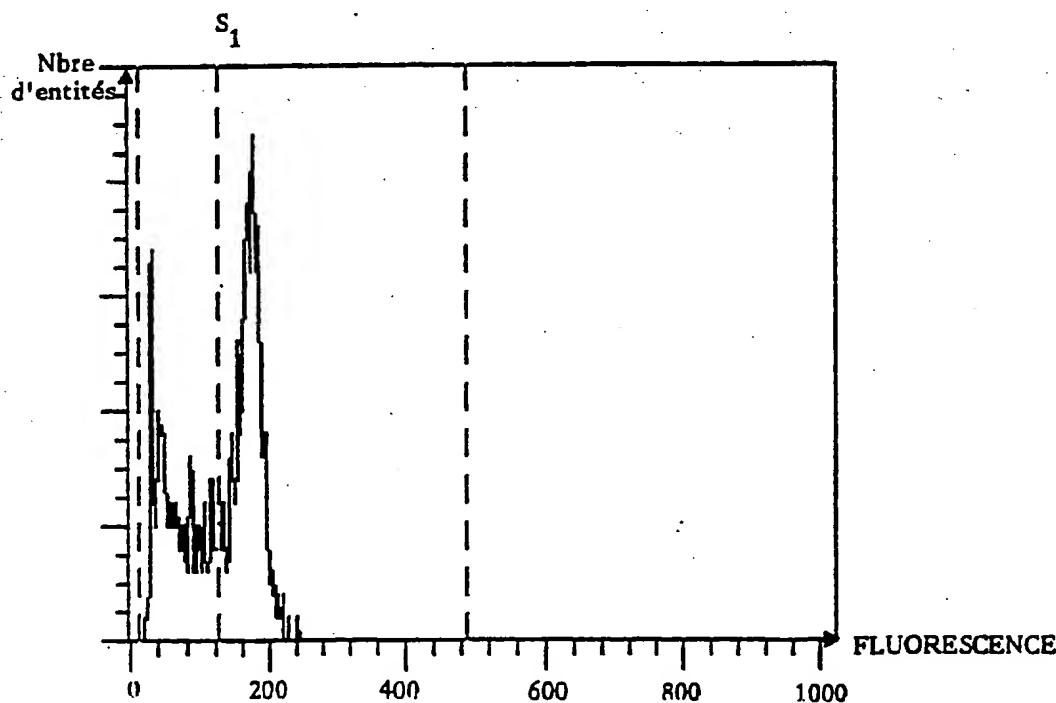


FIG 3



Office européen
des brevets

RAPPORT DE RECHERCHE EUROPEENNE

Numéro de la demande

EP 90 42 0212

DOCUMENTS CONSIDERES COMME PERTINENTS			
Catégorie	Citation du document avec indication, en cas de besoin, des parties pertinentes	Revendication concernée	CLASSEMENT DE LA DEMANDE (Int. Cl.5)
X	BIOLOGICAL ABSTRACTS, vol. 82, no. 12, 1986, pages AB-458, résumé no. 112110, Philadelphia, PA, US; M. HAGELTORN et al.: "Flow cytofluorometric characterization of bovine blood and milk leukocytes", & AM. J. VET. RES., 47(9): 2012-2016, 1986 * Résumé * ---	1	C 12 Q 1/04 G 01 N 33/50 G 01 N 33/04 G 01 N 1/30
A	IDEM ---	4	
Y	US-A-3 679 365 (K.B. WRIGHTMAN et al.) * Colonnes 1-2 * ---	1,6	
Y	CLINICAL CHEMISTRY, vol. 29, no. 2, février 1983, pages 290-296, Washington, US; D.C. SHELLY et al.: "Characterization of bacteria by mixed-dye fluorometry" * En entier * ---	1,6	
A	EP-A-0 268 766 (TOA MEDICAL ELECTRONICS CO., LTD) * Page 6, ligne 12 - page 8, ligne 3; revendications * ---	1,3,4,9 ,10	DOMAINES TECHNIQUES RECHERCHES (Int. CLS) C 12 Q G 01 N
A	BIOLOGICAL ABSTRACTS, vol. 86, no. 10, 1988, page AB-1153, résumé no. 108307, Philadelphia, PA, US; D. REDELMAN et al.: "Identification of inflammatory cells in bovine milk by flow cytometry", & CYTOMETRY 9(5): 463-468, 1988 * Résumé * -----	1,3	
Le présent rapport a été établi pour toutes les revendications			
Lieu de la recherche	Date d'achèvement de la recherche	Examinateur	
LA HAYE	11-06-1990	HITCHEN C.E.	
CATEGORIE DES DOCUMENTS CITES			
X : particulièrement pertinent à lui seul	T : théorie ou principe à la base de l'invention		
Y : particulièrement pertinent en combinaison avec un autre document de la même catégorie	E : document de brevet antérieur, mais publié à la date de dépôt ou après cette date		
A : arrêté-plan technologique	D : cité dans la demande		
O : divulgaion non écrite	L : cité pour d'autres raisons		
P : document intercalaire	& : membre de la même famille, document correspondant		

